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STUDY OF CRUDE EXTRACT FROM LAKOOCHA HEARTWOOD AGAINST ISOLATED FUNGUS

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ABSTRACT

The extract of heartwood and bark from lakoocha (Artocarpus lakoocha Roxb.) have pharmacollogical properties and antioxidant activities. These extracts have also been reported to exhibit antibacterial activity against a wide range of bacteria. However, there are a few studies on antifungal activity of lakoocha heartwood extract. This research aims to study the preliminary study of the effects of crude extract from A. lakoocha heartwood (CElaH) against the growth of fungi, which isolated from peanuts. A. lakoocha heartwood powder was extracted with 70% ethanol by Soxhlet extraction method to achieve crude extract. Fungi isolation was performed by direct plating method. The isolated fungus was white colony with dark-brown spores. It has conidia head and pyriform vesicle observed under a microscope. Therefore, Aspergillus sp. was expected as the isolated fungus. Effect of CElaH on growth of isolated fungus was determined by poison food technique under different concentrations (1-5 mg/ml). The highest percentage of fungal growth inhibition at 5 mg/ml CElaH on PDA and PDB mediums were 40.80% and 86.53%, respectively. The toxicity of the extract toward fungus was examined by transferring fungal disks from PDA mediums with 4 and 5 mg/ml CElaH to PDA mediums without CElaH. Results showed that fungus still alive and grow normally. Eventually, CElaH was natural plant extract, which tend to use an alternative compound to inhibit the growth of fungi.

KEYWORDS: Artocarpus Lakoocha Roxb, Lakoocha Heartwood, Soxhlet Extraction Method, Crude Extract & Poison Food Technique

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Original Article

INTRODUCTION

Background

The lakoocha (*Artocarpus lakoocha* Roxb.) is a tropical tree species of the family Moraceae (Joshee et al., 2002). It is widely distributed in the tropical regions of South and South-east Asia, including India, Srilanka, Nepal, Malaysia, Myanmar, Indonesia, Vietnam and Thailand. In Thailand, this plant is called as 'Ma-haad' (Singhatong et al., 2010). The trees are 6 to 9 m tall with leathery, large and deciduous leaves. Its fruits are pale yellow with pink tinge and sweet-sour pulp. The fruit shapes are almost round or irregular, 5 to 12 cm in diameter and have a velvety surface (Joshee et al., 2002). *A. lakoocha* is used as Thai traditional medicine for anti-inflammatory and an anti-skin aging agent (Hari et al., 2014). The dried aqueous extract prepared from its heartwood has been used as anthelmintic agent (Maneechai et al., 2008). The antioxidant activity of *A. lakoocha* heartwood extract was also reported (Singhatong et al., 2010).

A. lakoocha bark extract has been exhibiting antibacterial activity against different species of bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *B. pumilus*, *Proteus mirabilis* and *Klebsiella*

pneumoniae (Pandey and Bhatnagar, 2009). Additionally, the antimicrobial and antibiofilm activity of *A. lakoocha* extract against several strains of oral pathogens including Gram-negative and Gram-positive bacteria were reported (Teanpaisan et al., 2014). However, there are a few studies on antifungal activity of *A. lakoocha* heartwood extract. Therefore, this research was to primarily study the effects of crude extract from *A. lakoocha* heartwood (CElaH) against the growth of fungi, which was isolated from peanuts. Toxicity testing of CElaH against isolated fungus was also investigated.

METHODS

Isolation of Fungi from Peanuts

Fungus was isolated by direct plating method. Peanut samples were washed with tap water followed by 2-3 rinsing with distilled water. They were surface sterilized in 10 %(v/v) Clorox for 5 minutes and rinsed with sterile distilled water for 3 times. The seeds were then placed on PDA medium and incubated at 27±2°C for 7 days. To obtain pure culture, the mycelium which protruded from peanuts was transferred to new PDA medium and incubated at 27±2°C for 14 days.

Extraction of Crude Extract from *A. Lakoocha* Heartwood (Celah)

Five grams of *A. lakoocha* heartwood powder were extracted with 70% ethanol at 90°C for 10 h, using Soxhlet extractor. The entire extract was evaporated using rotary vacuum evaporator followed by drying in a hot air oven (60°C). The dry extract was weighed (1 g) and dissolved in 10 ml of 70% Ethanol to obtain 100 mg/ml CElaH. The CElaH was then kept in amber glass bottle at 4°C until next use.

Poison Food Technique

The effect of CElaH on growth of isolated fungi was examined by poison food technique. The CElaH was mixed with PDA medium to give concentration of 1, 2, 3, 4 and 5 mg/ml CElaH. PDA medium mixed with 70% Ethanol was used as a control. The fungal disks (5 mm diameter) were placed on PDA plates (one fungal disk per plate) and incubated at 27±2°C for 14 days. The diameters of fungal colonies were measured to calculate the percentage of growth inhibition (PGI) as follow:

$$PGI(\%) = \frac{A - B}{A} \times 100 \quad (1)$$

Where A = diameter of fungal colony on PDA medium with CElaH (cm), B = diameter of fungal colony on control plate (cm)

Inhibition of Fungal Growth by Celah in Liquid Culture

The CElaH was mixed with PDB medium to give concentration of 1, 2, 3, 4 and 5 mg/ml CElaH. PDB medium mixed with 70% Ethanol was used as a control. The fungal disks (5 mm diameter) were placed on the surface of PDB medium (one fungal disk per flask) and incubated with shaking at 100 rpm for 14 days at 27±2°C. The mycelium was harvested by filtration and dried at 105°C overnight in hot air oven, cooled in a desiccators to room temperature, and weighed. The dry weight of fungal mycelium was used to calculate PGI in liquid culture as equation (1), where A is dry weight of fungal mycelium in PDB medium with CElaH (g/l) and B is dry weight of fungal mycelium in control flask (g/l).

Toxicity Testing of Celah against Isolated Fungus

The fungal disks (5 mm diameter) from PDA medium with 4 and 5 mg/ml CElaH were transferred to new PDA medium without CElaH. They were incubated at 27±2°C for 14 days and the fungal growth were observed.

RESULTS

Isolation of Fungi from Peanuts

After isolation of fungi by direct plating method, only one fungus was obtained from peanut samples. On PDA, the isolated fungus was white colony with dark-brown spores (Figure 1a). It has conidia head and pyriform vesicle observed under a microscope at 400X (Figure 1b). Nyongesa et al. (2015) reported, genus *Aspergillus* is characterized by having a spore bearing structure called the conidia head. Therefore, isolated fungus was expected as *Aspergillus* sp.

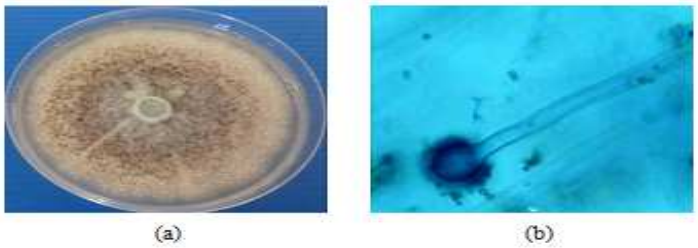

Figure 1: Colony of Isolated Fungus (A); Conidia Head and Pyriform Vesicle (B)

Inhibition of Fungal Growth by Celah

Poison food technique was performed to determined effect of CElaH on growth of isolated fungus under different concentrations (1-5 mg/ml). The results are shown in figure 2; diameters of colonies were measured to calculate PGI. The concentrations of CElaH in PDA, which gave high PGI were 4 mg/ml (37.51%) and 5 mg/ml (40.80%) (Table 1).

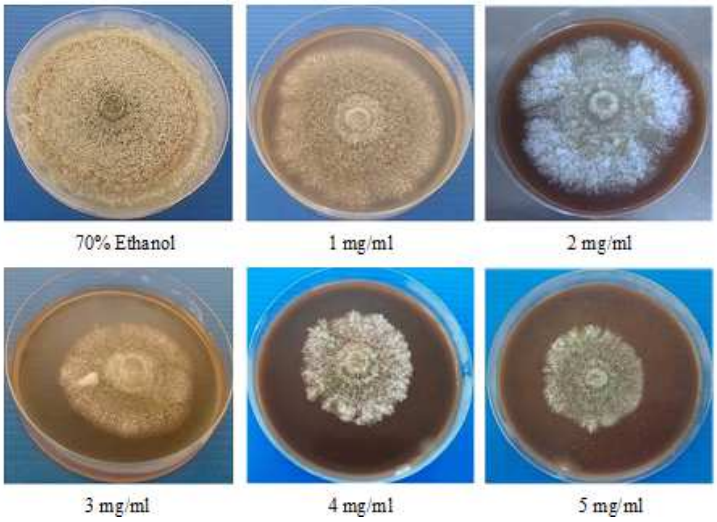

Figure 2: Colony of Isolated Fungus on PDA Medium with Different Concentrations of Celah and 70% Ethanol (Control)

Table 1: Effect of Celah on Growth of Isolated Fungus on PDA Medium

Concentrations of CElaH (mg/ml)	Diameter of Fungal Colony (cm)	PGI (%)
1	7.83 ± 0.21^{b}	8.21
2	7.50 ± 0.20^{b}	12.07
3	5.72 ± 0.10^{c}	32.94
4	5.33 ± 0.06^{c}	37.51
5	5.05 ± 0.18^{c}	40.80
Control (70% ethanol)	$^{8.53}$ 0.15^{a}	-

[a, b, c] Differences were considered statistically significant at P<0.05

In case of PDB medium with CElaH, fungal dry weight was decreased, when CElaH concentration increased from 1 to 5 mg/ml. The highest percentage of growth inhibition was 86.57% at 5 mg/ml CElaH, the following was 85.82% at 4 mg/ml CElaH (Table 2).

Table 2: Effect of Celah on Growth of Isolated Fungus in PDB Medium

Concentrations of CElaH (mg/ml)	Fungal Dry Weight (g/l)	PGI (%)
1	16.4 ± 0.02^{b}	38.81
2	15.9 ± 0.07^{b}	40.67
3	7.6 ± 0.30^{c}	71.64
4	3.8 ± 0.01^{d}	85.82
5	3.6 ± 0.00^{d}	86.57
Control (70% ethanol)	26.8 ± 0.09^{a}	-

[a, b, c, d] Differences were considered statistically significant at P<0.05

The results indicated that inhibition activity of the extract was concentration dependent (Dellavalle et al., 2011). *A. lakoocha* heartwood extract contained different totals of polyphenolic compounds (phenols, flavonoids and tannins) such as rutin, pyrogallol, gallic acid, resorcinol, quercetin, catechin and caffeic acid (Singhatong et al., 2010). These phenolic compounds were reported to have antifungal activity (Abad et al., 2007). Due to their structures, they can diffuse through the microbial membrane and penetrate into the cell, thus they can interfere the synthesis of ergosterol, glucan, chitin, proteins and glucosamine in fungi (Brul and Klis, 1999).

Toxicity Testing of Celah against Isolated Fungus

The isolated fungus from PDA medium with 4 and 5 mg/ml CElaH were cultured on PDA without CElaH. The results revealed that fungus still alive and grow normally; hence, the CElaH was not toxic to isolated fungus in this research. Since the toxicity of plant extract depends on active components that are extracted, which may be influenced by several factors such as age of plant, method of extraction, type of extracting solvent, plant species and extracts from different parts of plant (Alam et al., 2002; Mondall et al., 2009). For example, seed extract of *Azadirachta indica* could inhibit spore germination of *Fusarium oxysporum* f. sp. *vasinfectum* more than its root and leaf extract (Alam et al., 2002). Jayasinghe et al., 2004 reported that the *n*-butanol extract of the leaves of *A. nobilis* showed good fungicidal activity against *Cladosporium cladosporioides*, while the aqueous extract from *Phytolacca dodecandra* showed fungicidal activity against dermatophytes (Woldeamanuel et al., 2005).

CONCLUSIONS

In conclusion, the current study illustrated that CElaH has a potential to inhibit growth of isolated fungus, although it not showed toxicity to the fungus. However, CElaH is a natural plant extract, which possess active compounds with antifungal properties that may be used as biological fungicide.

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REFERENCES

1 *Abad, M. J., Ansuategui, M., & Bermejo, P. (2007). Active antifungal substances from natural sources. Arkivoc, 7, 116-145. doi: 10.3998/ark.5550190.0008.711*

2 *Alam, S., Akhter, N., Begum, M. F., Banu, M. S., & Islam, M.R. (2002). Antifungal activities (In vitro) of some plant extracts and smoke on four fungal pathogens of different hosts. Pakistan Journal of Biological Sciences, 5, 307-309. doi: 10.3923/pjbs.2002.307.309*

3 *Brul, S., & Klis, F. M. (1999). Mechanistic and mathematical inactivation studies of food spoilage fungi. Fungal Genetics and Biology, 27(2), 199-208. doi: 10.1006/fgbi.1999.1149*

4 *Dellavalle, P. D., Cabrera, A., Alem, D., Larrañaga, P., Ferreira, F., & Rizza, M. D. (2011). Antifungal activity of medicinal plant extracts against phytopathogenic fungus Alternaria spp. Chilean Journal of Agricultural Research, 71(2), 231-239. doi: 10.4067/S0718-58392011000200008*

5 *Hari, A., Revikumar, K. G., & Divya, D. (2014). Artocarpus: a review of its photochemistry and pharmacology. Journal of Pharma Search, 9(1), 7-12.*

6 *Jayasinghe, L., Balasooriya, B. A. I. S., Padmini, W. C., Hara, N., & Fujimoto, Y. (2004). Geranyl chalcone derivatives with antifungal and radical scavenging properties from the leaves of Artocarpus nobilis. Photochemistry, 65(9), 1287-1290. doi: 10.1016/j.phytochem.2004.03.033*

7 *Joshee, N., Bastola, D. R., Agrawal, V. P., & Yadav, A. K. (2002). Lakoocha: A multipurpose tree of warm climate. Trends in new crops and new uses, ASHS Press, Alexandria, VA, 405-406.*

8 *Maneechai, S., Likhitwitayawuid, K., Sritularak, B., Palanuvej, C., Ruangrungsi, N., & Sirisa-ard, P. (2009). Quantitative analysis of oxyresveratrol content in Artocarpus lakoocha and 'Puag-Haad'. Medical Principles and Practice, 18, 223–227. doi: 10.1159/000204354*

9 *Mondall, N. K., Mojumdar, A., Chatterje, S. K., Banerjee, A., Datta, J. K., & Gupta, S. (2009). Antifungal activities and chemical characterization of Neem leaf extracts on the growth of some selected fungal species in vitro culture medium. Journal of Applied Sciences and Environmental Management, 13(1), 49-53.*

10 *Nyongesa, B.W., Okoth, S., & Ayugi, V. (2015). Identification key for Aspergillus species isolated from maize and soil of Nandi County, Kenya. Advances in Microbiology, 5, 205-229. doi: 10.4236/aim.2015.54020*

11 *Pandey, A., & Bhatnagar, S. P. (2009). Preliminary Photochemical screening and antimicrobial studies on Artocarpus lakoocha Roxb. Ancient Science of Life, 28(4), 21–24.*

12 *Singhatong, S., Leelarungrayub, D., & Chaiyasut, C. (2010). Antioxidant and toxicity activities of Artocarpus lakoocha Roxb. Heartwood extract. Journal of Medicinal Plants Research, 4(10), 947-953. doi: 10.5897/JMPR10.133*

13 *Teanpaisan, R., Senapong, S., & Puripattanavong, J. (2014). In vitro antimicrobial and antibiofilm activity of Artocarpus lakoocha (Moraceae) extract against some oral pathogens. Tropical Journal of Pharmaceutical Research, 13(7), 1149-1155. doi: 10.4314/tjpr.v13i7.20*

14 Woldeamanuel, Y., Abate G., & Chryssanthou, E. (2005). In vitro activity of Phytolacca dodecandra (Ended) against dermatophytes. Ethiopian medical journal, 43(1), 31-34.